A DISSERTATION
FOR THE DEGREE OF MASTER

Intraocular Pressure Measurement with a Rebound Tonometer (TonoVet®) in Normal Pigeons

정상 비둘기 안구에서 TonoVet® 안압계를 이용한 안압 측정

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ABSTRACT

The purpose of this study was to evaluate the applicability of a rebound tonometer (TonoVet®) in pigeon eyes and to establish normal reference intraocular pressure (IOP) values in healthy pigeons. Twenty eyes of euthanized pigeons were used for the calibration of TonoVet® and 48 eyes of 24 adult pigeons for the reference IOP. First, IOP of pigeon eyes *ex vivo* were measured using the 'd' and the 'p' modes of the TonoVet® and compared with manometric IOP values from 5 to 80 mmHg. Then, to establish normal reference values, IOP was measured from clinically normal pigeons in vivo. Both the 'd' and the 'p' modes of the TonoVet® showed a strong linear correlation with the manometric IOP (R^2 = 0.996 and 0.991, respectively). The obtained regression formulas were $y = 0.431x_1 + 2.154$ and $y =$

$0.330x_2 - 0.673$, respectively (x_1, 'd' mode of TonoVet®; x_2, 'p' mode of TonoVet®; y, manometric IOP). The 'd' and the 'p' modes steadily underestimated the actual IOP by approximately 1/2 and 1/3, respectively. Therefore, the formula obtained through the 'd' mode was applied to obtain reference values. The calibrated IOP of normal pigeon eyes was 19.5 ± 4.4 mmHg. The actual IOP could be calculated using the presented formula. Considering the limitations of the 'p' mode, it would be more appropriate to use the 'd' mode. Therefore, the TonoVet® rebound tonometry under 'd' mode is a reliable method for measuring IOP in pigeons.

Keywords: intraocular pressure, rebound tonometry, pigeons, Columbiformes, reference values, linear regression analysis

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CONTENTS

INTRODUCTION

Pigeons have a long history interacting with humans, and there are several domestic breeds (Harlin and Wade, 2009). Although pigeons have been used in various fields such as an avian model for research, studies on pigeons are scarce, especially regarding their intraocular pressure (IOP). Measurement of IOP is an important part of ophthalmic examinations and an essential process for diagnoses of glaucoma or uveitis (Reuter *et al*., 2010). Therefore, a reliable and precise method of IOP measurement for pigeons is required (Park *et al*., in press).

Applanation and rebound tonometers are widely used in veterinary fields. The most commonly used tonometers are the TonoPen XL® (applanation tonometer) and the TonoVet® (rebound tonometer) (Dubicanac *et al*., in press). The TonoVet® is designed for animals but has developed an internal calibration curve only for the measurement of IOP in dogs and cats (the 'd' mode) and horses (the 'h' mode) (Jeong *et al*., 2007). The tonometer should be calibrated because characteristics of cornea vary among between different species (Reuter *et al*., 2010). In a previous study, the TonoVet® was found to be less accurate in pigeons than a TonoPen XL®, compared to actual manometers, but more tolerable for IOP measurements in pigeons (Görig *et al*., 2005). Recent studies have widely used the TonoVet® and the TonoPen XL® in other animals (Delgado *et al*., 2014; Dubicanac *et al*., in press; Lewin and Miller, in press; McLellan *et al*., 2013). It has been necessary to obtain a regression formula and research an accurate method for measuring the actual IOP of living pigeons.

The purpose of this study was to determine a tolerable and accurate method for measuring IOP in pigeons (*Columba livia* var. *domestica*).

MATERIALS AND METHODS

1. Experimental animals

Twelve pigeons (20 eyes) euthanized for other research purposes were used for manometric calibration and 24 clinically normal pigeons (48 eyes) were surveyed to establish normal reference of IOP values. Healthy adult pigeons of undetermined sex were used in this study. Prior to the experiments, full ophthalmic examinations of all pigeons, including slit-lamp biomicroscopy (Keeler PSL One Portable Slit Lamp; Keeler Ltd., Windsor, UK) and direct ophthalmoscopy (WA 11720; Welch Allyn, New York, USA), were performed to confirm the absence of ocular diseases. All ophthalmologic examinations and IOP measurements were performed by the same experienced examiner (JG) to reduce examiner bias. Three successive measurements per eye were obtained, and only values within the range of allowable standard deviation per the manufacturer ($\leq$ 2.5 mmHg) were recorded. This was indicated by “no bar” or “bar down” on the display.

2. Manometric calibration of TonoVet®

Pigeons were fixated in a standing posture using a stereotaxic instrument (Model 900; Kopf Instruments, Tujunga, CA, USA). Lateral canthotomy was performed and the anterior chamber was cannulated at the 3 or 9 o'clock position of the limbus with a 30-gauge needle (Fig. 1) connected to a system consisting of a pressure monitoring kit (Transpac IV Monitoring Kit; ICU Medical Inc., San Clemente, CA, U.S.A.) with a transducer, polyethylene tubes, monitoring cable (Transpac Reusable Cable; Hospira Inc., Lake Forest, IL, U.S.A.), and a monitor (CARESCAPE Monitor B650; GE Healthcare Finland Oy, Finland). The digital manometer was used to detect instantaneous IOP changes and to ensure that there was no leakage from the anterior chamber. A three-way stopcock was used to connect the anterior chamber to the manometer and a NaCl solution. Artificial tears (Refresh Plus®; Allergan, Irvine, CA, USA) were frequently topically applied to prevent the cornea from desiccation. Accuracy and reliability of the device were checked against a mercury manometer before conducting the experiments. For measurements, the ‘d’ and the ‘p’ modes (for dogs and for unspecified, respectively) of the TonoVet® (Icare, Tiolat, Helsinki, Finland) were used. IOP values measured using the TonoVet® were compared with manometric IOP values from 5 to 80 mmHg by using a height-adjustable stand with the NaCl solution reservoir. IOP measurements were performed when IOP was considered constant without fluctuation on the digital manometer. Measurements were performed in 5 mmHg steps from 5 to 40 mmHg, which is considered clinically significant, and in 10 mmHg increments from 40 to 80 mmHg. Animal care and procedures were approved by the Institutional Animal Care and Use Committees of the Seoul National University (SNU-160812-5).

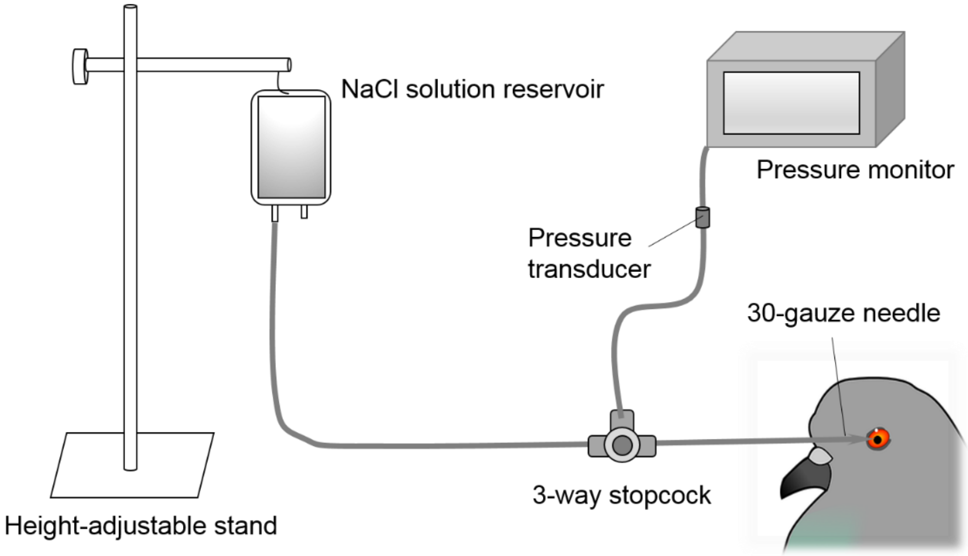

Fig. 1. Experimental setting for the manometric calibration of the TonoVet®. The head was fixated with the stereotaxic instrument in a standing posture. The anterior chamber was cannulated using a 30-gauge needle. The 3-way stopcock connected the anterior chamber to a height-adjustable NaCl solution reservoir and a digital manometer.

3. Determination of normal pigeon IOP

Each pigeon was held in a relaxed posture and the wings were gently pressed against the body (Fig. 2). The pigeons were measured after waiting approximately 3 minutes for stabilization. All measurements were taken between 6 pm and 10 pm. IOP was measured three times using the ‘d’ mode. Values were averaged and the obtained mean value was applied to the regression formula to calculate the actual IOP.

Fig. 2. Rebound tonometry using the TonoVet® in a pigeon (*Columba livia* var. *domestica*) which was held in a relaxed posture.

4. Statistical analyses

A paired student's t-test was used to compare the IOP measurements between left and right eyes. Data are presented as mean ± standard deviation (SD). The regression formula of the tonometry versus manometry was obtained via linear regression analysis. Tonometric and manometric values were compared using Bland-Altman plots. Statistical analyses were conducted using SPSS 23 software (IBM SPSS Statistics®; Arm onk, NY, USA) for Windows.

RESULTS

1. Manometric calibration of TonoVet®

The post-mortem IOP of 12 pigeons (20 eyes) was measured. All pigeon eyes were confirmed to have no abnormalities. Comparison of manometric and tonometric IOP measurements showed significant differences between the two methods ($P < 0.001$) over the entire pressure range. At 5 mmHg, the values measured using the 'd' mode were comparable to the manometrically obtained values despite the fact that the difference was statistically significant. The 'd' mode constantly underestimated the actual IOP by approximately 1/2 from 10 to 80 mmHg, whereas the 'p' mode constantly underestimated approximately 1/3 of the actual IOP. Although both the 'd' and the 'p' modes of the TonoVet® tend to underestimate the actual manometric values, both modes showed a strong linear correlation between tonometric and manometric values (Fig. 3). The regression formulas for the 'd' mode and the 'p' mode were $IOP_{mano} = 0.439 \times IOP_d + 2.059$ ($F = 2740.453$, $r^2 = 0.996$, $P < 0.001$) and $IOP_{mano} = 0.330 \times IOP_p - 0.673$ ($F = 1124.182$, $r^2 = 0.991$, $P < 0.001$), respectively. Bland-Altman plots were used to show the difference between tonometry and manometry. The values gradually differed in the high IOP range in both the 'd' and the 'p' modes (Figs. 4 and 5). Manometric IOP was greater than tonometric IOP within the whole pressure range. Nevertheless, most plots were distributed within 95% limits, confirming that the two measurement methods were well correlated.

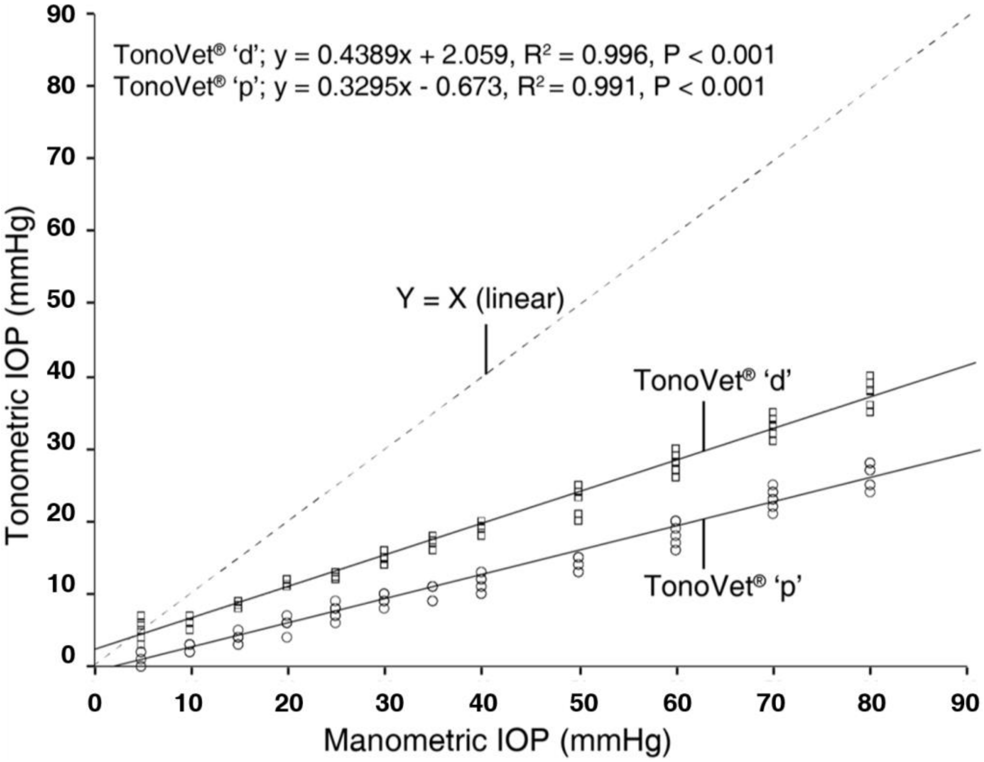

Fig. 3. All IOP readings measured using the ‘d’ mode (open square) and the ‘p’ mode (open circle) of the TonoVet®. Some points are superimposed.

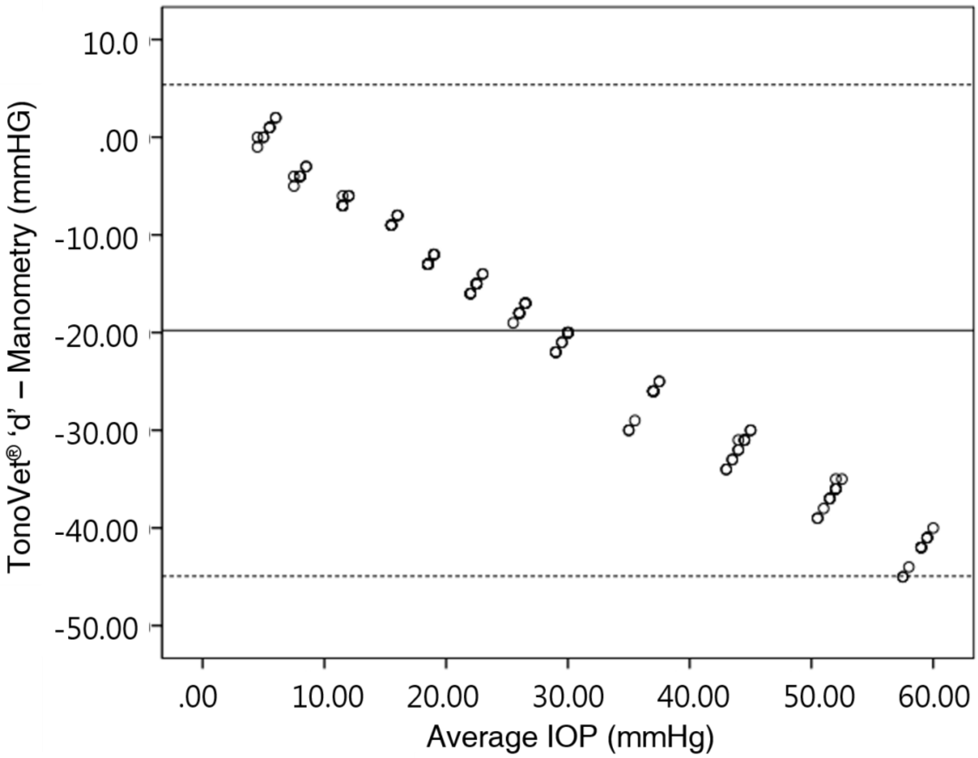

Fig. 4. Bland-Altman plot for comparison of the IOP measured using the 'd' mode of TonoVet® and the manometer. Dashed lines indicate 95% confidence intervals. Some points are superimposed.

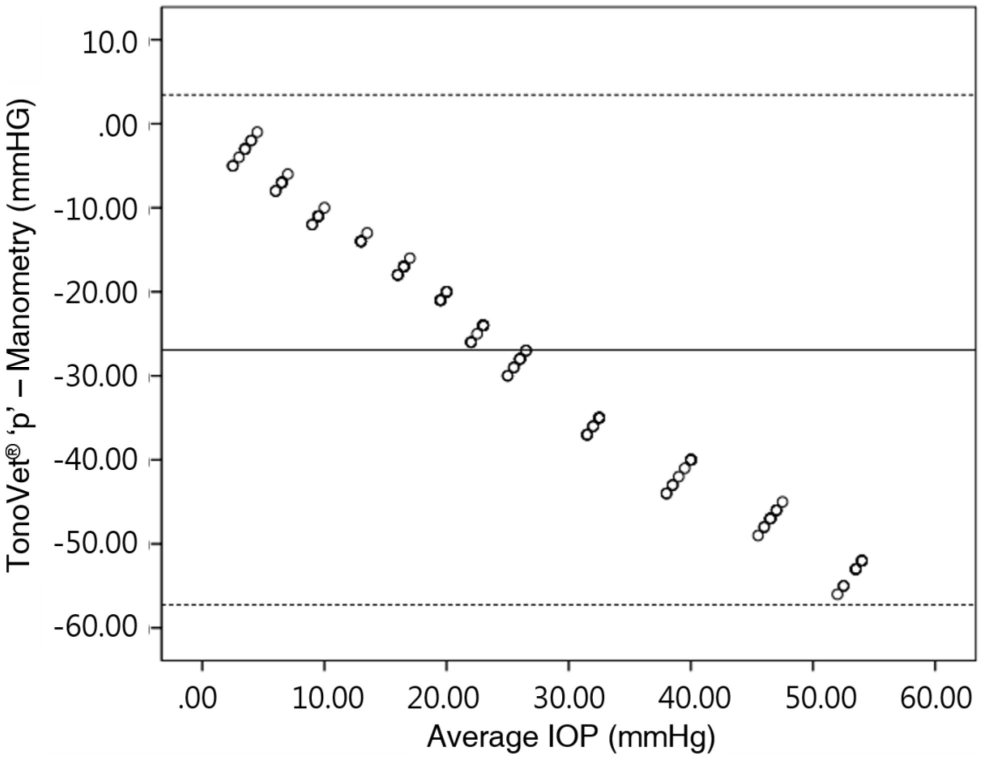

Fig. 5. Bland-Altman plot for comparison of the IOP measured using the 'p' mode of TonoVet® and the manometer. Dashed lines indicate 95% confidence intervals. Some points are superimposed.

2. Determination of normal pigeon IOP

Pigeons tolerated the procedure well because of the fast and reduced stress-inducing approach of rebound tonometry during the IOP measurement (24 pigeons, 48 eyes). Ophthalmic examinations revealed no evidence of ophthalmic diseases in all pigeons. There was no difference in IOP between left and right eye (P = 0.381 in the 'd' mode and P = 0.556 in the 'p' mode, respectively). Therefore, both eyes were analyzed without distinction.

Both the 'd' and 'p' modes were used for the measurements. However, only the values measured using the 'd' mode were analyzed for calibration because the 'p' mode severely underestimated the actual IOP. The mean IOP value measured using the 'd' mode was 10.6 ± 1.9 mmHg, the median was 11 mmHg, and the range was 7~13 mmHg. The calculated mean IOP was 19.5 ± 4.4 mmHg, the median was 20.4 mmHg, and the range was 11.3~24.9 mmHg (Table 1).

Table 1. IOP values of 48 eyes in 24 clinically normal pigeons

	Median	Range	Mean ± SD
TonoVet® 'd' IOP (mmHg)	11.0	7.0-13.0	10.6 ± 1.9
Calculated IOP (mmHg)	20.4	11.3-24.9	19.5 ± 4.4

IOP = intraocular pressure; SD = standard deviation.

DISCUSSION

Normal IOP references have been established in domestic animals, such as dogs (Gelatt and MacKay, 1998; Knollinger *et al*., 2005; Leiva *et al*., 2006; Miller *et al*., 1991a; Priehs *et al*., 1990), cats (Miller *et al*., 1991b; Rusanen *et al*., 2010), horses (Knollinger *et al*., 2005), calves (Tofflemire *et al*., 2015), and sheep (Ghaffari *et al*., 2011), and in laboratory animals such as rats (Goldblum *et al*., 2002; Moore *et al*., 1993), rabbits (Ma *et al*., 2016; Pereira *et al*., 2011), and mouse lemurs (Dubicanac *et al*., in press). IOP references for some avian species have also been reported in previous studies (Harris *et al*., 2008; Jeong *et al*., 2007; Stiles *et al*., 1994). In this study, the normal IOP reference data of the pigeon was evaluated.

The most commonly used tonometers in the veterinary field are the rebound tonometer, TonoVet®, and the applanation tonometer, TonoPen XL® (Dubicanac *et al*., in press). Previous studies have suggested differences in IOPs measured in birds between the TonoVet® rebound tonometer and the TonoPen XL® applanation tonometer (Harris *et al*., 2008; Jeong *et al*., 2007). In addition, the basic settings of the TonoVet® are applicable only to specific species such as dogs, cats and horses (Dubicanac *et al*., in press). Therefore, to obtain the actual IOP from new species, tonometry should be calibrated using manometry (Dubicanac *et al*., in press).

For various reasons, the TonoPen XL® was not used for the measurements in this study. As mentioned in a previous study, the TonoPen XL® causes stress, high systemic blood pressure, and additional compression to the eyeball, which could result in a high value of IOP (Dubicanac *et al*., in press). Also, the diameter of the cornea of the pigeon is less than 9 mm, which is unsuitable for the application of the TonoPen XL® (Willis and Wilkie, 1999). The tip of the TonoPen XL® (1.0 mm transducer tip) is too large for small eyes and could cause reflex blinking. In addition, topical anesthesia is necessary and requires an extended duration up to 10 minutes. Furthermore, focusing on applied pressure, contact area, and body position can result in a high failure rate (Dubicanac *et al.*, in press).

In this study, the TonoVet® was selected for the measurements because it is known to cause minimal response and reflex blinking when the probe contacts the cornea, and no additional force is needed to fixate the eye. In addition, the measuring time is short approximately 30 seconds; the TonoVet® is appropriate for the small cornea of the pigeon, and topical anesthesia is unnecessary. Moreover, the measurement standardized and routinized quickly (Dubicanac *et al.*, in press).

In a previous study, the TonoVet® was reported to be less accurate than the TonoPen XL® in pigeons based on the absolute difference between the mean IOPs measured with the tonometers and the mean IOPs recorded using the manometer (Görig *et al.*, 2005). However, by using the obtained regression formula in this study to compensate for the difference, the TonoVet® demonstrated accurate measurement of IOP in pigeons.

The TonoVet® is a rebound tonometer that has three modes: ‘d’, ‘h’, and ‘p’ modes. The ‘d’ mode is applied to dogs and cats, the ‘h’ mode is used for horses, and the ‘p’ mode is for measurements in unspecified animals. In the present study, both the ‘d’ mode and the ‘p’ modes showed a strong linear correlation and had a tendency to underestimate the actual IOP. However, the ‘d’ mode was used to obtain the reference IOP because there were some limitations in measuring with the ‘p’ mode. The ‘p’ mode values showed a larger difference from actual IOP values than the ‘d’ mode values. The ‘d’ mode measured approximately 50% of the actual

IOP, whereas the 'p' mode was only able to measure about a third of the actual IOP. Moreover, in some eyes at the 5 mmHg step, the IOP, as measured by the ‘p’ mode, was 0 mmHg.

The previous study has found that the average IOP values of pigeons in the 'd' mode was 11.7 ± 1.6 mmHg (10 pigeons, 20 eyes) (Park *et al.*, in press). In this study, the mean value of the pigeon IOP measured in the 'd' mode was 10.6 ± 1.9 mmHg (24 pigeons, 48 eyes). Converting this into the regression formula, the reference IOP of pigeons was actually 19.5 ± 4.4 mmHg.

Direct measurements of IOP through an invasive method can only be used for calibration of non-invasive methods (Reuter *et al.*, 2010). Considering the welfare of animals, post mortem measurement was conducted. Measurements after enucleation can result in lack of the orbital boundaries, which can affect the IOP measurement by changing the surrounding orbital structures (Reuter *et al.*, 2010). Thus, only a lateral canthotomy was performed without enucleation, such that unnecessary procedures did not affect the IOP. Using the stereotaxic instrument, it was possible to avoid any other interference to the measurement of IOP.

In determining the reference IOP, there was a lack of information, such as sex and age. There were also insufficient pigeon populations. In addition, the diurnal variation was not investigated. In this study, IOP was measured only in clinically normal pigeons. Further studies on pigeons with ophthalmic diseases, such as glaucoma or uveitis, are needed to be carried out.

CONCLUSIONS

The results of this study suggest that the TonoVet® is a well-tolerated and accurate instrument for measuring the IOP of pigeons. The 'd' mode of the TonoVet® rebound tonometry is proper in measuring the IOP in normal pigeons. The 'd' mode constantly underestimated the actual IOP by about 50%. The actual IOP can be achieved by converting tonometric values using the regression formula.

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국 문 초 록

정상 비둘기 안구에서 TonoVet® 안압계를 이용한 안압 측정

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본 연구에서는 TonoVet® 안압계를 비둘기 (*Columba livia*) 에 적용하여 실용성을 평가하고 건강한 비둘기에서의 정상 안압 범위를 확인하고자 하였다. 12마리의 안락사 된 비둘기의 20개의 안구에 압력계와 높이 조절이 가능한 수액대를 연결하여 안압을 조절해가며 안압계로 측정하였다. 5-40 mmHg 까지는 5 mmHg 단위로, 40-80 mmHg 까지는 10 mmHg 단위로 안압을 측정하였고, 각 단계마다

3회씩 안압을 측정하였다. 이를 선형회귀분석으로 분석하여 회귀분석식을 얻었다. 그 후 살아있는 24마리의 정상 비둘기의 48개 안구에서 안압을 측정하였고 이를 앞에서 구한 회귀분석식에 적용하여 실제 안압값을 구하였다. 그 결과 TonoVet® 의 'd' 모드와 'p' 모드는 모두 압력계와 강한 선형 상관관계를 보였다. 얻어진 회귀식은 각각 'd' 모드는 $y = 0.431x_1 + 2.154$, 'p' 모드는 $y = 0.330x_2 - 0.673$ 였다. (x_1, TonoVet®의 'd' 모드 측정값; x_2, TonoVet®의 'p' 모드 측정값; y, 압력계 측정값). 'p' 모드는 실제 값을 너무 낮게 측정하여 'd' 를 이용해 정상 비둘기의 안압을 측정하였고, 그 평균값인 10.6 ± 1.9 mmHg 를 회귀식에 적용하면 19.5 ± 4.4 mmHg 이다. 따라서 본 연구에서 얻어진 회귀식을 이용하면 정확한 비둘기 안구 압력을 계산할 수 있으며, TonoVet®의 'd' 모드 가 비둘기 압력 측정에 더 적합한 것으로 판단된다.

주요어: 안압, 압평 안압계, 선형회귀분석, 비둘기

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